Genome-wide genotyping of acute myeloid leukemia with translocation t(9;11)(p22;q23) reveals novel recurrent genomic alterations

Chromosomal translocations of the *mixed-lineage leukemia (MLL)* gene are common genetic events in acute leukemias. In acute myeloid leukemia (AML), the translocation t(9;11)(p22;q23) [subsequently referred to as t(9;11)], resulting in the MLL-MLLT3-fusion protein, is the most common translocation involving *MLL*. Translocation (9;11) can be found in both *de novo* and therapy-related AML (t-AML) and the current WHO classification separates these two subtypes into distinct categories.[1] *De novo* and t-AML generally differ in terms of treatment response and survival with therapy-related cases being associated with inferior outcome and abnormal and more complex cytogenetics.[1,2] However, little is known about the biological and clinical difference between *de novo* and therapy-related *MLL-MLLT3*-rearranged AML.

The exposure to chemo-/radiation therapy as it occurs in t-AML is hypothesized to alter the number of copy number alterations (CNAs) that could potentially reflect biological differences between these AML subtypes. Furthermore, cooperating genetic events have been suggested to be required for *MLL*-leukemogenesis, since knock-in mice that express the *MLL-MLLT3*-fusion gene under control of the MLL promoter develop AML with a leukemia onset that ranges between four months to over one year.[3]

To explore number and type of cooperating genetic lesions in AML with t(9;11), we performed SNP-array based genotyping of 40 diagnostic leukemia samples from *de novo* and t-AML patients (*de novo*, n=22; t-AML, n=16; unknown, n=2) (*Online Supplementary Table S1*). Intra-individual germline DNA from remission bone marrow or peripheral blood was available for paired analysis in 15 cases. In order to minimize false positive calls, lesions from the unpaired cohort were considered truly somatically required, only if they were found to be altered in at least one sample from the paired cohort. SNP-array (GeneChip Human Mapping 6.0; Affymetrix)-based genotyping and data analysis were performed as previously described.[4] Raw data were made publicly available at the NCBI Gene Expression Omnibus (series accession number GSE46745).

Paired analysis of the 15 cases revealed few somatic genetic alterations with only 1.73 CNAs per case (range 0-13). Deletions were more common than gains (*de novo*: 0.75 losses/case *vs.* 0.38 gains/case; t-AML: 2.29 losses/case *vs.* 0.14 gains/case, all mean) and 53% of all cases lacked any detectable CNAs (*de novo*: 50%; t-AML: 57%). Notably, no significant difference was observed in the mean number of CNAs between *de novo* and t-AML (*de novo*: 1.13/case *vs.* t-AML: 2.43/case; P=0.78). Only one single t-AML case exhibited 13 CNAs (case MLL#3; *Online Supplementary Table S2*). These findings clearly distinguish t-AML with t(9;11) from other t-AML entities that frequently exhibit additional chromosomal alterations,[2] although the statistical power of this analysis might be limited by the small collection of paired samples. The relatively small number of CNAs in *de novo* cases is consistent with prior reports on distinct types of *de novo* AML[5] and *MLL-AF4*-rearranged acute lymphoblastic leukemia (ALL)[6] but stands in clear contrast to most other ALL subtypes.[7]

Despite the small number of CNAs identified, we were able to detect several recurrent lesions (Table 1 and *Online Supplementary Tables S2 and S3*). The most common cytogenetic event was trisomy 8 detected in 20% of the 40 cases (*de novo*: 27%; t-AML: 6%; P=0.21) (Table 1 and Figure 1A). Besides focal recurrent deletions and gains adjacent to the genes involved in t(9;11) (Table 1), we identified two novel minimally altered regions (MARs) at chromosomal bands 7q36.1-q36.2 (loss: 5%; *de novo*: 5%; t-AML: 6%) and 13q21.33-q22.1 (gain: 5%; *de novo*: 5%; t-AML: 6%) (Table 1). Both regions contain genes whose alterations may cooperate with the translocation-encoded MLL-MLLT3-fusion protein to induce overt leukemia. Del(7q) is a recurrent alteration frequently seen in t-AML and complex karyotype AML but also occurs in other AML subtypes such as the Core-Binding Factor (CBF)-AMLs.[4,8] The identified MAR at 7q36 contains only 6 genes (Table

Table 1. Recurrent copy number alterations (CNAs) in 40 *de novo* and therapy-related acute myeloid leukemia cases with t(9;11).

Lesion type and cytoband	Loc. start*	Loc. end*	MAR size (Kb)	N. total lesions at locus	N. *de novo* lesions at locus	N. therapy-related lesions at locus	Gene and miRNA symbols (if less than 10)**
del(7)(q36.1-q36.2)	151,675,439	152,604,848	929	2	1	1	***MLL3***, *FABP5L3, CCT8L1,* ***XRCC2***, *ACTR3B, ARP11*
trisomy 8	21,242	146,268,972	whole chromosome	8	6	1	
gain(13)(q21.33-q22.1)	72,097,687	73,119,202	1025	2	1	1	*C13orf37, C13orf34,* ***DIS3***, *KIAA1008, PIBF1,* ***KLF5***
gain(13)(q12.12)	22,537,900	22,791,657	254	2			SGCG
gain(13)(q12.12-q12.13)	24,083,858	24,478,645	395	2			ATP12A, RNF17, CENPJ, TPTE2C1
t(9;11)-breakpoint associated lesions							
gain(9)(p22.1)	19,204,401	19,545,583	341	2	1	1	*DENND4C, RPS6, ACER2, ASAH3L, SCL24A2*
del(11)(q23.3)	117,861,238	117,946,853	86	4	3	1	***MLL***, *BC039603, TTC36, TMEM25, C11orf60, IFT46*

*MAR: minimally altered region. *hg18. Genes previously reported as oncogenes/tumor suppressor genes and/or listed within the Network of Cancer Genes (NCG 3.0) are highlighted in bold.*

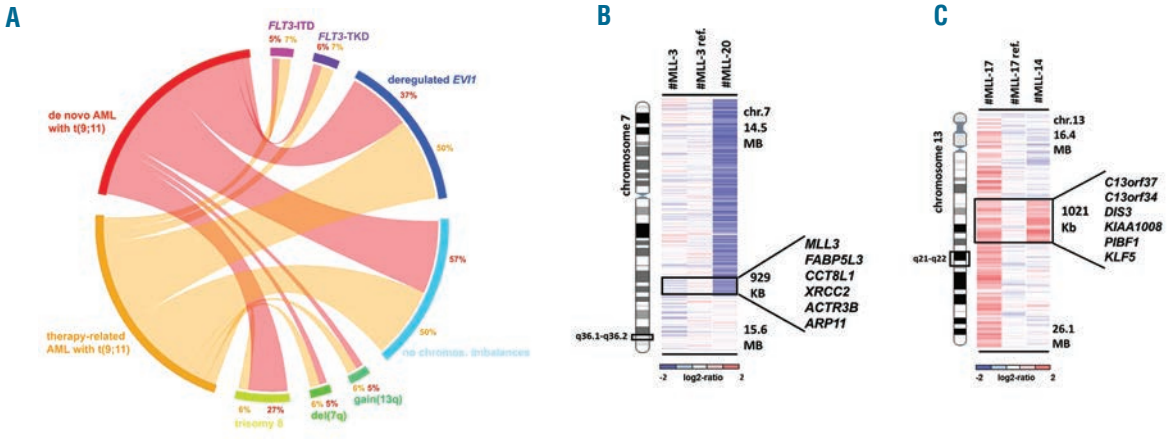

Figure 1. (A) Circos-plot illustration of recurrent secondary genetic alterations in 22 *de novo* (red) and 16 t-AML cases (orange) with t(9;11). Frequencies of respective lesions are indicated in percent [deregulated *EVI1* = over-expressed *EVI1*; no chromos. imbalances = cases lacking any CNAs]. (B and C) Heat map of recurrently altered regions in t(9;11)-positive AML. (B) Log_2-ratio SNP copy number data of deletion 7q36.1-q36.2 involving *MLL3* showing one large and one micro-deletion [ref.: paired normal control; minimally altered region (MAR) containing 6 genes as indicated]. (C) Gain of 13q21.33-q22.1 involving *KLF5* and *DIS3* [ref.: paired normal control; MAR is defined by case #MLL-14 including 6 genes].

1 and Figure 1B) and is partly overlapping with the MAR that we previously described at 7q36.1 in CBF-AMLs.[4] Of note, the overlapping region contained only the *MLL3* gene. *MLL3* is one of five members of the *MLL* gene family that encode H3K4 methyltransferases with homology to the Drosophila-trithorax gene and have all been implicated in cancer.[9] The gain(13)(q21.33-q22.1) affected six genes (Table 1 and Figure 1C). Of these, the two genes *DIS3* and *KLF5* were previously implicated in oncogenesis. *KLF5* is a member of the *krüppel-like factor* transcription factor family critically involved in regulation of cell proliferation and differentiation.[10] It promotes cell proliferation in various tissue types such as gastrointestinal epithelial cells and embryonic stem cells[11] and is found to be over-expressed in gastric carcinoma.[12] To explore the role of *KLF5* gene expression in *MLL-MLLT3*-rearranged AML, we performed Affymetrix HGU133 plus 2.0 microarray analysis in 20 cases (10 cases with and 10 cases without available SNP-array data). Cases were dichotomized into high and low *KLF5* expression cases based on the median expression of *KLF5*. In the retrospective survival analysis of this highly selected sample collection, high *KLF5* expression was associated with inferior overall survival (*Online Supplementary Table S4 and Figure S1*). Inactivation mutations of *DIS3*, another gene located within the gain(13q), were recently reported in relapsed AML.[13] Notably, in the total cohort of 40 cases, no single region of CN-LOH was identified. This finding is in clear contrast to other AML subtypes where CN-LOH is more common[14] and suggests that the mechanism of CN-LOH seems not to play a critical role in *MLL-MLLT3*-rearranged AML. Sequence analysis of selected AML candidate disease genes revealed mutations in *FLT3* (ITD, 2 of 36, 6%; TKD: 3 of 29, 10%; *NPM1*: 2 of 31, 7%) at a low incidence, whereas mutations in *IDH1/2* (0 of 29), *DNMT3A* (0 of 19), *TET2* (0 of 15), and *NRAS* (0 of 6) were absent. In contrast, deregulated *EVI1* expression was a frequent event affecting 53% of t(9;11)-positive AML patients.

This finding is consistent with recently reported data demonstrating *EVI1* to be essential for tumor growth in a subset of *MLL-MLLT3*-rearranged AML.[15] However, with regard to number or type of CNA, we did not find any substantial differences between *EVI1* high and low expression cases (*Online Supplementary Tables S2 and S3*). Based on this observation, we cannot propose an increase in genomic complexity as the consequence of the interaction between *MLL* and *EVI1*. In summary, our study demonstrates that CNAs occur at a very low frequency in t(9;11)-positive AML. However, we discovered novel recurrently altered regions that point to potential cancer disease genes. With regard to CNAs, we found no differences in number or type of lesions between *de novo* and t-AML with t(9;11), calling into question the current WHO classification which separates them into different categories. Further clinical outcome data are needed to address this issue.

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